

Colony PCR

- Pick one colony with a sterile tip and elute it in 100 μ L ddH₂O or medium
- Store the colony in 4°C while colony PCR is running
- One reaction mix contains
 - 10 μ L 5x buffer
 - 2 μ L MgCl₂ (25mM stock)
 - 1 μ L dNTPs
 - 0.5 μ L primer mix (prefix/suffix primer or sequencing primers)
 - 35.25 μ L ddH₂O
 - 0.25 μ L GoTaq polymerase (Promega)
 - 1 μ L template
- PCR program:
 - Start: 3min at 98°C
 - 30 cycles of:
 - 30s at 98°C
 - 30s at 55°C
 - 30s / 1kb template, 72°C
 - Finish: 5min at 72°C
- Gel electrophoresis: check fragment size
- Plate the correct colony

